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Original Articles

Potential invasiveness by non-indigenous macrozoobenthos in the secondary hydrographic system of a temperate-climate river catchment

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ABSTRACT

In the context of biological invasions, freshwater aquatic invertebrates represent one of the most successful groups of organisms. At the same time, the secondary hydrographic system of a river catchment acts as a preferential pathway for the dispersion of Non-Indigenous Species (NIS) to nearby ecosystems. The present study investigates the macrozoobenthos assemblage inhabiting 13 representative semi-natural streams of the secondary hydrographic system of the southern course of the River Ticino (Lombardy, Northern Italy). The level of biocontamination of the habitats under study was assessed by the Site-specific BioContamination Index (SBCI), which was paired with a preliminary screening of the NIS that may cause ecological and economic impacts at the sites under study using the recently-developed Aquatic Species Invasiveness Screening Kit (AS-ISK) tool. The SBCI classified one stream as 'high class', one as 'good class', two as 'moderate class', seven as 'poor class', and the remaining two as 'bad class'. Six macrozoobenthic NIS were identified, namely the amphipod *Gammarus roeselii*, the gastropods *Physella acuta*, *Potamopyrgus antipodarum* and *Pseudosuccinea columella*, the bivalve *Corbicula fluminea* and the limpet *Ferrissia fragilis*. Based on the AS-ISK outcomes, the most potentially invasive species were *C. fluminea* followed by *P. columella*, both of which were categorised as 'high risk'. Also, the potential invasiveness of the above species increased after accounting for climate change predictions, as did that for all other species assessed, hence regardless of their corresponding risk level. By combining the results of the SBCI index with those from AS-ISK, a first step is provided towards the identification and refinement of potential management strategies aimed to reduce the impacts of NIS in the RAA, whilst accounting for predicted effects of climate change.

1. Introduction

Freshwater ecosystems are subject to different types of pressure, including water pollution, agricultural and industrial over-exploitation, as well as alteration and loss of habitat (Cardoso and Free, 2008). Another important stressor for these ecosystems is represented by the invasion of Non-Indigenous Species (NIS), which can cause serious ecological and economic impacts, ultimately leading to 'biological pollution' or 'biocontamination' (Elliott, 2003; Arbačiauskas et al., 2008). Although many NIS are introduced into new environments, most these species are unable to survive, whereas a few others successfully establish themselves and become Invasive Alien Species (IAS). These have been defined as "a subset of established NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an adverse effect on biological diversity, ecosystem functioning, socio-economic values and/or human health in invaded regions" (Olenin et al., 2010, p. 7). Yet, it is generally difficult to quantify the ecological impacts of NIS. This is because of their generally long-term effects on the ecosystem, which require appropriately time-scaled and accurate research studies (Olenin et al., 2007).

In the context of biological invasions, freshwater aquatic invertebrates represent one of the most successful groups of organisms. This is due to their biological traits, which are typical of successful NIS (Bij de Vaate et al., 2002), and to their ability to colonise both lentic and lotic ecosystems. In this respect, the secondary hydrographic system of a river catchment acts as a preferential pathway for the dispersion of NIS to nearby ecosystems (Rewicz et al., 2015; Grabowski et al., 2017). In general, the secondary hydrographic system plays a multi-functional role by acting as a refuge for many invertebrate species, and usually consists of an intricated web of semi-natural streams of different size, some of which originate by superficial waters, whereas others derive from natural terrace springs or directly from the main river course. Almost all of these streams are characterised by running waters, pebble–sandy bottoms and abundant aquatic plants, and are

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surrounded by riparian vegetation. However, in areas characterised by high anthropogenic impact, natural freshwater ecosystems are often small sized, isolated and surrounded by agricultural or urbanised areas. Yet, such ecosystems still play an important role in connecting different natural areas by favouring genetic exchange between populations, hence acting as a 'sink of biodiversity'. Overall, the secondary hydrographic system can be considered as a crucial stepping-stone habitat for several organisms, despite an overall underestimation of its ecological value (Havel et al., 2005).

This study focuses on the macrozoobenthos assemblage inhabiting the secondary hydrographic system of the southern course of the River Ticino (Lombardy, Northern Italy), which is located in a temperate-climate catchment subject to high anthropogenic impact (Marchetti, 2002; Ingegnoli, 2015; Garzoli et al., 2014) and to increasingly disrupted trends in rainfall events (Brunetti et al., 2000). The specific objectives are to: (i) define the level of biocontamination of these lesser-studied environments using a specific index of biocontamination, and (ii) provide a preliminary evaluation of the NIS that may cause ecological and economic impacts in the study area by the adoption and implementation of a recently-developed risk screening tool for aquatic species. It is expected that the outcomes of this study will represent a first step towards the successful management of the aquatic environment of the River Ticino secondary hydrographic system.

2. Methods

2.1. Study area and sampling

In total, 13 semi-natural streams were selected across an area of ≈150 km^2 in the southern course of the River Ticino valley (namely, eleven streams on the right bank and two on the left bank of the main river channel: Table 1, Fig. 1), which will be hereafter defined as the Risk Assessment Area (RAA). All of the studied streams are characterised by sandy-to-gravel substrata with moderately running waters and with the bottom partially covered by a thick layer of leaf detritus and macrophytes. From June to September 2015, three random subsamples of macrozoobenthos were collected in each stream along a 10 m transect consisting of homogeneous habitat, which reflects the entire characteristics of the semi-natural streams under study, using a modified net (950 µm mesh size) equipped with a square frame (22 × 23 cm, for an area of 0.0506 m^2). Upon collection, samples were preserved in 70% ethanol and then transferred to the laboratory for later identification at the species level and counting.

2.2. Risk screening

The level of biocontamination in each of the streams under study was assessed using the Site-specific BioContamination Index (SBCI: Arbačiauskas et al., 2008). The SBCI is derived from two indices, namely the Abundance Contamination Index (ACI) and the Richness Contamination Index (RCI). The ACI is computed as the ratio between the number of specimens of non-indigenous taxa and the total number of specimens in a sample; the RCI is the ratio between the total number of non-indigenous orders and the total number of orders. All non-indigenous taxa are considered in this index, regardless of their actual status of invasiveness or potential invasiveness. The resulting SBCI range of values is then divided into five classes from a minimum of 0 ('no contamination') to a maximum of 4 ('severe contamination'). The lowest quality ('bad') class is obtained when NIS represent either more than half of the detected orders or when their abundance exceeds 50% of the individuals. Notably, the five SBCI classes match the five ecological quality classes identified in the Common Implementation strategy for the EU Water Framework Directive (2000/60/EC) (European Community, 2000, 2003) (Table 2). Additionally, the ecological quality of the semi-natural streams was defined by means of the Italian version of the Extended Biotic Index (EBI: Woodiwiss, 1978), namely the "Indice Biotico Esteso" (IBE) as modified by Ghetti (1986) for Italian water courses.

The Aquatic Species Invasiveness Screening Kit (AS-ISK) (Copp et al., 2016; available for free download at www.cefas.co.uk/nns/tools/) was used to identify potentially invasive species with respect to the RAA under investigation. AS-ISK is a generic replacement of the Freshwater Invertebrate Invasiveness Screening Kit (FI-ISK: Tricarico et al., 2010). It consists of 49 questions on the biogeography/historical and biology/ecology aspects of the taxon under assessment, and these questions comprise the Basic Risk Assessment (BRA). In addition, six Climate Change Assessment (CCA) questions (specific to AS-ISK only: Copp et al., 2016) require the assessor to predict how future climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. For each question, the

Fig. 1. Study area: the River Ticino catchment in south-west Lombardy (Northern Italy).

Table 1
Labels and WGS84 coordinates of the 13 semi-natural streams of the secondary hydrographic system of the southern course of the River Ticino (Lombardy, Northern Italy).

Semi-natural streams	Code	Coordinates WGS84
Canale Scavizzolo	SCA	N 45°17′15.8″; E 08°56′06.1″
Fosso Canalino	CAN	N 45°16′08.9″; E 08° 59′ 25″
Roggia Nuova	RN	N 45° 16′ 07.9″; E 08° 56′ 33.7″
Roggia Grignina	RG	N 45° 15′ 43.9″; E 08° 57′ 01.9″
Roggia Cerro	RC	N 45° 15′ 16.1″; E 08° 57′ 02.9″
Roggia Vecchia	RV	N 45° 14′ 46.1″; E08°56′ 06.7″
Canale Venara	VEN	N 45°11′44.2″; E 09°04′10″
Canale Gaviola	GAV	N 45°11′26.9″; E 09°04′03.2″
Cavo Moraschino	MOR	N 45°10′48.4″; E 09°05′22.2″
Roggia Vernavola	VER	N 45°10′17.5″; E 09°12′18.1″
Colatore Gravellone	GRA	N 45°10′14″; E 09°10′12.1″
Canale Rotta	RO	N 45°09′41.8″; E 09°08′26.2″
Roggia Grande	GR	N 45°09′07.2″; E 09°07′41.3″

Table 2
Assessment of the Site-specific BioContamination Index (SBCI) based on the results of the Abundance Contamination Index (ACI) and Richness Contamination Index (RCI). The five SBCI classes are: 0 (no contamination, high status), 1 (low contamination, good status), 2 (moderate contamination, moderate status), 3 (high contamination, poor status) and 4 (severe contamination, bad status). Modified from Arbačiauskas et al. (2008).

SBCI	ACI				
RCI	None	0.01–0.10	0.11–0.20	0.21–0.50	> 0.50
None	0				
0.01–0.10		1	2	3	4
0.11–0.20		2	2	3	4
0.21–0.50		3	3	3	4
> 0.50		4	4	4	4

assessor must provide a response, justification and level of confidence, and the taxon assessed eventually receives both a BRA and a BRA + CCA (composite) score (respectively ranging from −20 to 68 and −32 to 80). As in FISK, AS-ISK scores < 1 suggest that the NIS is unlikely to become invasive in the RAA (hence, categorised as 'low risk'), whereas higher values will classify the NIS as posing either a 'medium risk' or a 'high risk' of becoming invasive. The distinction between medium and high risk level depends upon definition of a 'threshold' value. This is either obtained through an RAA-specific 'calibration' based on a representative number of taxa or, in case of small sample sizes, 'borrowed' from other studies (see Copp, 2013; Hill et al., 2017). Finally, the ranked levels of confidence (1 = low, 2 out of 10 chances; 2 = medium, 5 out of 10; 3 = high, 8 out of 10; 4 = very high, 9 out of 10) associated with each response mirror the confidence rankings recommended by the International Programme on Climate Change (see also Copp et al., 2016) and are combined into a Confidence Factor (CF) ranging from 0.25 to 1 (e.g. Tarkan et al., 2017a).

2.3. Statistical analyses

Differences in the density of macrozoobenthic NIS amongst streams were tested by permutational multivariate analysis of variance (PERMANOVA) based on a one-factor design with Stream as the random factor. Analysis was performed in PERMANOVA+ for PRIMER v6, with normalisation of the data and using a Bray-Curtis dissimilarity measure, 9999 unrestricted permutations of the raw data (Anderson et al., 2008), and with statistical effects evaluated at $\alpha = 0.05$. Briefly, the advantage of PERMANOVA compared to traditional parametric analysis of variance is that the stringent assumptions of normality and homoscedasticity, which proved very often unrealistic when dealing with ecological datasets, are 'relaxed' considerably.

Given the relatively small size of the RAA combined with the limited number of observed NIS, it was not possible in the present study to carry out a RAA-specific calibration of the AS-ISK outcome scores. At the same time, the only calibrated study by Tricarico et al. (2010) for freshwater invertebrates in Italy, albeit providing a FI-ISK-based threshold to distinguish medium- and high-risk species, is not transferable to AS-ISK due to the changes in several questions and the resulting differences in the overall scoring system in the latter tool (Copp et al., 2016; see also Glamuzina et al., 2017; Tarkan et al., 2014 vs. Tarkan et al., 2017a). For this reason, preliminary thresholds for BRA = 36 and BRA + CCA = 38 obtained from a global (i.e. worldwide) assessment of aquatic invertebrates providing for a representative sample size (Copp and Vilizzi, unpublished data) were used in the present study as a first attempt to distinguish between medium- and high-risk NIS.

3. Results

According to the "Indice Biotico Esteso" (IBE), Roggia Vernavola was classified in the II–III classes of ecological quality (slightly-moderately polluted), the other five semi-natural streams in class III (moderately polluted), six of them in class IV (heavily polluted), and only Roggia Nuova in class V (very heavily polluted) (Table 3).

During the study period, 17% of the total macrozoobenthic organisms collected were NIS, with the most abundant being the gastropod *Potamopyrgus antipodarum*, the amphipod *Gammarus roeselii*, the bivalve *Corbicula fluminea*, and the gastropods *Physella acuta*, *Ferrissia fragilis* and *Pseudosuccinea columella* (Fig. 2a). However, these six most abundant NIS were not found in all of the semi-natural streams under study. Specifically, the most frequently-occurring NIS were *P. antipodarum* and *G. roeselii*, which were collected respectively in nine and ten out of the 13 streams investigated; conversely, *P. columella* occurred only in one stream (Fig. 2b).

In the streams Canale Gaviola, Roggia Cerro, Roggia Grignina and Roggia Nuova, the most abundant NIS were *P. antipodarum* and *G. roeselii*, and proportionally these were also consistently more abundant compared to the native species. The highest densities of NIS were recorded in Roggia Grignina (43%) and Roggia Cerro (42%); on the contrary, the semi-natural streams with the lowest NIS densities were Roggia Vernavola (≈4%) and Cavo Moraschino (no NIS recorded) (Fig. 3). Notably, these differences in density amongst streams were statistically significant (PERMANOVA: $F^{\#}_{1,12} = 2.39$, $P = 0.001$; # = permutational).

The macrobenthic community of Roggia Grande had the highest level of abundance contamination (ACI = 0.75), whereas the lowest value was recorded in Canale Moraschino (0) (Fig. 4). With regard to the RCI, the highest value was recorded in Roggia Gaviola (0.5) and the lowest again in Canale Moraschino (0) (Fig. 4). As a result, the SBCI classified Canale Moraschino as 'high class' quality (0) and Canale Scavizzolo as 'Good class' (1). Also, there were two streams with SBCI = 2 ('moderate class'), seven streams with SBCI = 3 ('poor class'),

Table 3
Results of the application of the Indice Biotico Esteso (IBE, Ghetti, 1986). SU: Systematic Units; IBE: index values; QC: Quality Classes.

Semi-natural streams	Code	SU	IBE	QC
Canale Scavizzolo	SCA	7	6	III
Fosso Canalino	CAN	12	7	III
Roggia Nuova	RN	3	3	V
Roggia Grignina	RG	6	5–4	IV
Roggia Cerro	RC	6	5–4	IV
Roggia Vecchia	RV	13	7	III
Canale Venara	VEN	8	6	III
Canale Gaviola	GAV	8	5	IV
Cavo Moraschino	MOR	4	4	IV
Roggia Vernavola	VER	16	8–7	II–III
Colatore Gravellone	GRA	6	5–4	IV
Canale Rotta	RO	14	7	III
Roggia Grande	GR	7	5	IV

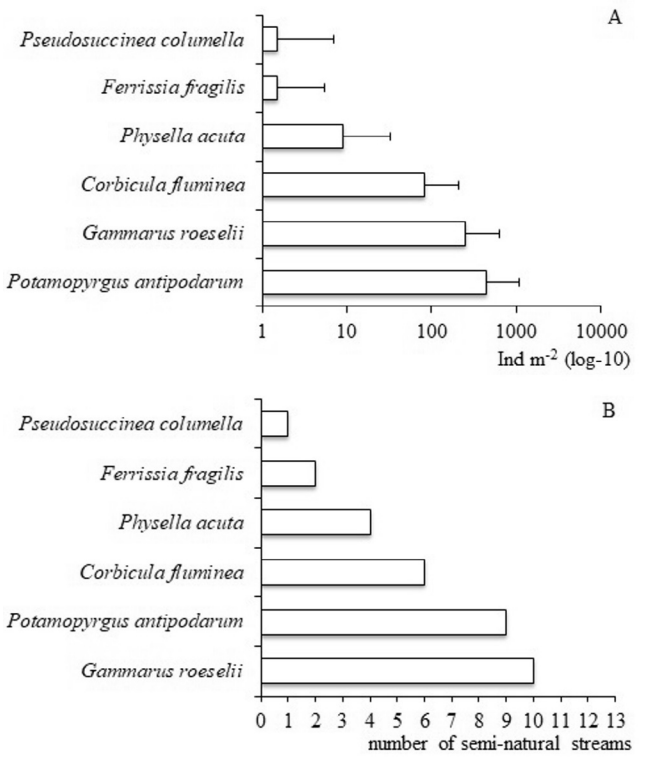

Fig. 2. Mean density and standard deviation (logarithmic scale Ind m^{-2}) (A) and occurrence of NIS (B) collected in 13 semi-natural streams of the southern course of the River Ticino.

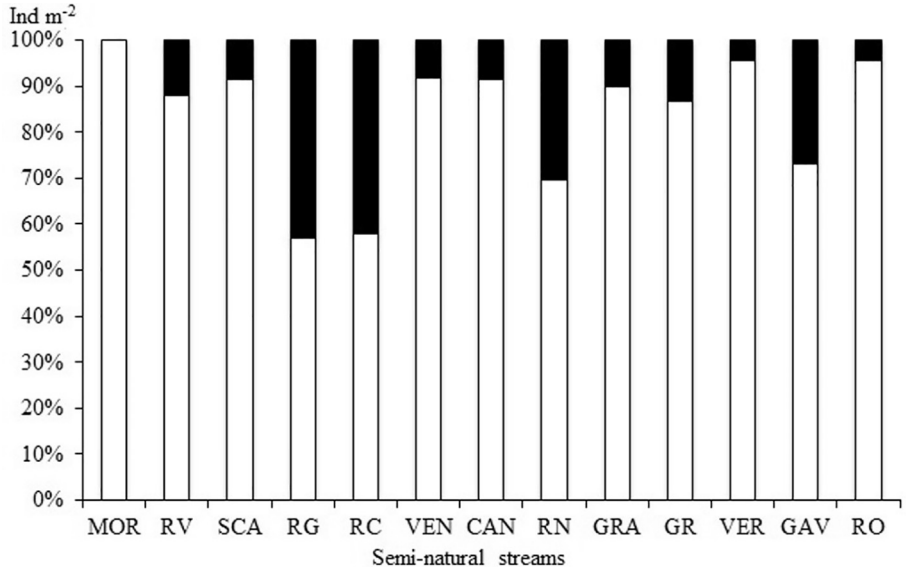

Fig. 3. Percentage of NIS (black) and native species (white) collected in each of the13 semi-natural streams of the southern course of the River Ticino. For labels of the semi-natural streams see Table 1.

and another two streams with SBCI = 4 ('bad class') (Fig. 3b). Using the SBCI, which accounts for the presence and density of these six NIS, the RAA was classified as having a medium/poor level of biocontamination (mean SBCI ± standard error = 2.6 ± 0.12).

Based on the AS-ISK outcomes, the most potentially invasive species in the NIS assemblage for the RAA under study were *C. fluminea* followed by *P. columella*, both of which were categorised as 'high risk' (Table 4). The most abundant species *P. antipodarum* ranked third in both BRA and BRA + CCA scores and was categorised as 'medium risk' together with *G. roeselii*, *P. acuta* and *F. fragilis* (in descending order of BRA and CCA scores). For all species assessed, the CCA section resulted in a positive value (either 4 or 8), which increased the corresponding BRA scores. Finally, the CF ranged from a minimum of 0.65 for *P. columella* to a maximum of 0.82 for *P. acuta*.

4. Discussion

In this first risk screening study of the 13 main semi-natural streams of the secondary hydrographic system of the southern course of the River Ticino, six macrozoobenthic NIS in total were identified.

Except for *G. roeselii* and *P. columella*, the rest of the NIS assemblage was represented by species quite common across the study area (Ruffo and Stoch, 2005), and despite the relatively low number of NIS in the community, these were found to reach high densities in some streams. However, similar to native species, the distribution and abundance of macrobenthic NIS may be influenced by a combination of several environmental factors (e.g. water temperature, dissolved oxygen, sediment granulometry), thereby affecting the value of both the SBCI and IBE even though a clear ecological relationship between these two indices has not been identified. For example, in the Roggia Vernavola stream, only a few NIS with relative low densities were collected; whereas, in other sections of the same stream characterised by similar environmental conditions compared to those reported in the present study, the density of *C. fluminea* was higher (Paganelli et al., in press). However, despite the statistically significant differences, there is not clear explanation as to why some streams hosted more NIS than others, especially given that these originated from the main course of the River Ticino and, also showed similar environmental preferences (D. Paganelli, pers. obs.). Also, it is difficult to know exactly when and how these NIS entered the RAA due to an overall lack of data from the literature on these freshwater ecosystems.

According to AS-ISK, the most invasive species categorised as 'high risk' for the RAA was *C. fluminea*. This is a medium-sized bivalve native to south-east Asia that is now widespread in many European countries

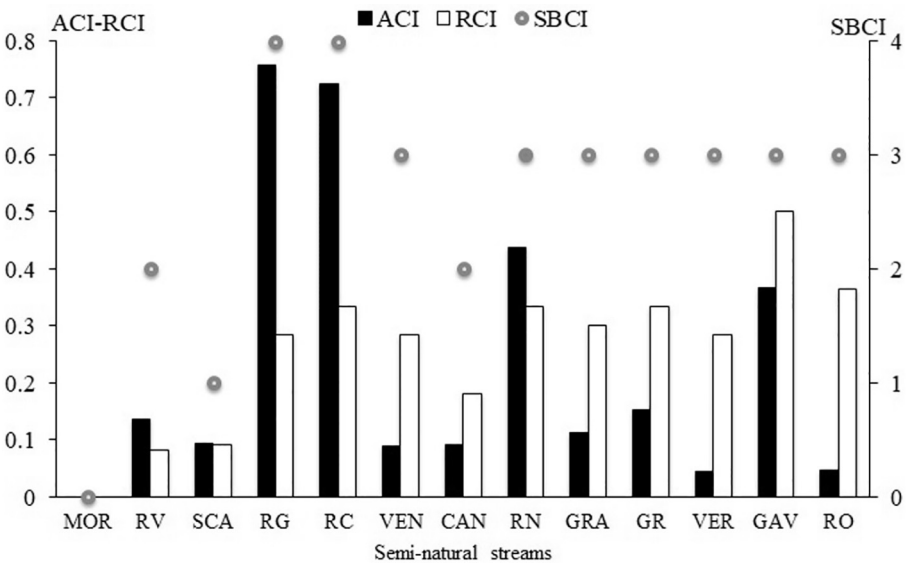

Fig. 4. Results of the application of the Abundance Contamination Index (ACI), the Richness Contamination Index (RCI) and the Site-specific BioContamination Index (SBCI). For labels of the semi-natural streams see Table 1.

Table 4
AS-ISK Basic Risk Assessment (BRA) and Climate Change Assessment (CCA) scores, and level of risk and confidence (CF) for the six NIS collected.

Species	Biogeography/Historical	Biology/Ecology	Climate change	BRA	Risk level	BRA + CCA	Level of risk	CF
Corbicula fluminea	11.5	27	8	38.5	High	46.5	High	0.72
Pseudosuccinea columella	13	23	8	36	High	44	High	0.65
Potamopyrgus antipodarum	2	24	8	26	Medium	34	Medium	0.78
Gammarus roeselii	4	17	4	21	Medium	25	Medium	0.71
Physella acuta	-1	18	8	17	Medium	25	Medium	0.82
Ferrissia fragilis	0.5	15	4	15.5	Medium	19.5	Medium	0.76

(including Italy) as well as in other continents (Aldridge et al., 2012). The high invasiveness of this Asiatic clam is thought to be due not only to its tolerance to environmental factors such as low oxygen levels, increased water temperatures and elevated concentrations of nitrogen compounds (Sousa et al., 2008; Bódis et al., 2012), but also to its high spreading potential. In this respect, adult individuals of *C. fluminea* can actively move against the water flow, whereas their larvae can exploit various types of vectors (i.e. fishes, aquatic birds, as well as human beings) to move upstream (McMahon, 1999; Prezant and Chalermwat, 1984; Schmidlin and Baur, 2007). Moreover, this species is characterised by both sexual and asexual reproduction, and whilst sexual populations are restricted to the native Asian areas, the asexual reproductive mode is widely found in populations from North America and Europe, where *C. fluminea* invasion has been based on the successful clonal dispersion of genetically undiversified populations (Pigneur et al., 2014). High abundances of *C. fluminea* have led to a decrease in molluscan bivalve biodiversity due to competition with native species for space and resources (Global Invasive Species Database, 2017), and besides its ecological impacts this species has also been responsible for negative economic impacts, including the clogging of pipes and loss of amenity values (Sousa et al., 2014).

The second species also categorised as 'high risk' was *P. columella*. This is a fusiform-shell medium-sized (height up to ≈2 cm) pulmonate gastropod from North America that has been recently introduced to several European countries including Italy (Turner et al., 1998; Falkner et al., 2001; Anderson, 2004; Reischütz and Reischütz, 2004; Bank, 2005). One of the species' main vectors of introduction is represented by anthropogenic activities, as demonstrated by its first introduction to Italy, where it was discovered in a botanical garden and whose presence was traced back to the importation of ornamental aquatic plants (Cianfanelli et al., 2007). Despite its reported ability to adapt to most environments (Prepelitchi et al., 2011), *P. columella* still has rather limited distribution in Europe, probably due to unfavourable environmental conditions. This is evidenced under warmer climates, such as those found in Australia (Smith and Stanisic, 2006), Cuba (Gutiérrez et al., 2001) and Hawaii (Cowie, 1998), where the species is known to have spread rapidly. However, as highlighted by the corresponding CCA score, the distribution and abundance of *P. columella* could ultimately increase as a result of (predicted) global warming conditions, in which case its competition for food with native gastropods could also further increase, with foreseeable impacts on biodiversity. Finally, *P. columella* is considered to be a vector of the parasitic flatworm *Fasciola gigantica* (Trematoda), which affects livestock in Europe, Asia and Africa, and is therefore being targeted by control programmes aimed at its eradication (Cordeiro and Bogan, 2012).

The third species in invasiveness ranking categorised as 'medium risk' was *P. antipodarum*. This is a small prosobranch gastropod that was introduced into Europe from New Zealand at the end of the 19th century (Smith, 1889). Nowadays, *P. antipodarum* has colonised all European countries including Italy, where it is regarded as the most widespread non-indigenous prosobranch (Berner, 1963; Favilli et al., 1998, Bodon et al., 1995). Its invasiveness is due to various life-history traits such as euryoeciousness, parthenogenetic reproduction, and dispersal with fish re-stocking and anthropogenic transport, all of which facilitate its rapid spread (Alonso and Castro-Diez, 2008). This gastropod can reach very high densities, as reported in the present study and elsewhere (Adam, 1942; Lucas, 1959; Réal, 1973; Falniowski, 1979). Due to its ability to consume large amounts of resources, *P. antipodarum* can alter ecosystem dynamics, compete with and displace native invertebrates, and negatively influence higher trophic levels (Kerans et al., 2005; Hall et al., 2006; Alonso and Castro-Diez, 2008; Davidson et al., 2008; Cowie, 1998). By way of example, in the USA high densities of *P. antipodarum* are believed to have been responsible for negative interactions with native macroinvertebrates (Richards and Cazier Shinn, 2004; Kerans et al., 2005; Brown et al., 2008); and in Mont Saint-Michael Bay (western France), the species is known to dominate the local macrobenthic community by representing 80% of the identified gastropods (Gerard et al., 2003). Similarly, *P. antipodarum* made up to 83% of the mollusc community in a reservoir near an industrial area in Poland (Lewin and Smolinski, 2006). Another ecological impact of *P. antipodarum* relates to the fish fauna, which the species can directly affect by representing a poor and mostly undigestible food source (Vinson and Baker, 2008). Further, *P. antipodarum* has fouling potential due to its ability to pass through water pipes and emerge from domestic traps to the point of clogging entire irrigation systems (Ponder, 1988; Zaranko et al., 1997). *Potamopyrgus antipodarum* has also been found to be infected by the blood fluke *Sanguinicola* sp. in Europe and therefore may contribute to the spread of this parasite to new areas (Gerard and Le Lannic, 2003). Once established, eradication of this species becomes difficult in most cases, if not impossible. Prevention of its introduction and containment of existing populations is therefore paramount to minimise its spread and related impacts, especially in view of its likely further expansion throughout its introduced range (Global Invasive Species Database GISD 2015).

The fourth (medium-risk) species was *G. roeselii*. This is a slow-spreading amphipod gammarid native to the Balkan Region and recorded for the first time in the Ticino River basin in 2015 (Paganelli et al., 2015). This species relies on different vectors for spreading, possibly including aquatic birds or unintentional introductions by anthropogenic activities (Pandolfi et al., 2017). Moreover, *G. roeselii* reaches higher densities in small rivers and can survive out of water for relatively long periods (Meijerinmg, 1991; Starry et al., 1998; Pöckl, 2003; Mayer et al., 2012; Pandolfi et al., 2017). Its potential impacts mainly result from its possible competition with the native amphipod *Echinogammarus stammeri*, with which it shares habitat and food resources (i.e. aquatic plants and leaf debris: Paganelli et al., 2016, 2017). However, these impacts may not be very pronounced, as indicated by the relatively lower score achieved by the species in the present AS-ISK based assessment. Furthermore, *G. roeselii* has no economic value and apparently does not exert any direct impacts on ecosystem services. It is also argued that expected climate change conditions may not prove much advantageous to this species at least in the RAA under investigation, except for possible global warming-related alterations (e.g. flash-floods leading to increased connection between the semi-natural streams of the catchment).

The second last ranking (medium-risk) NIS was *Physella acuta*. This is a medium-size basommatophoran pulmonate that is common and abundant in lotic and lentic water bodies. This species was introduced into Europe from North America (Taylor, 2003), with its first report in Italy dating as back as the 18th century (Issel, 1866). According to

Manganelli et al. (2000), its introduction was one of the causes for the gradual rarefaction of the indigenous basommatophore *Physa fontinalis* (Linnaeus). *Physella acuta* is currently distributed across a wide range of natural and man-made water bodies (mainly, irrigation channels, dams, and streams in or near towns) throughout Europe, and in Italy it occurs in all 20 regions, where it is often found in large populations inhabiting highly-polluted water bodies (Feliksiak, 1939; Saraceni, 1971; Moretti et al., 1979; Melone, 1981). Overall, this species is very adaptable and can be considered a serious pest. However, because *P. acuta* forages mainly on epiphytic vegetation and macrophytes (as opposed to other gastropods exploiting algal cover or phytobenthos), competition with other gastropods is thought to be minimal (Semenchenko et al., 2008). *Physella acuta* is hermaphroditic and can reproduce uni- and bi-parentally, and after 8 weeks reaches adult maturity and is ready to reproduce (Sánchez-Argüello et al., 2008). Also, this NIS can survive well under temporary harsh conditions of extreme water temperatures and pollution (Semenchenko et al., 2008). *Physella acuta* has been widely introduced and can be transported by birds and human-related activities. It also represents an important intermediate host for many parasites (Toledo et al., 1999, 2000; Faltýnková, 2005; Faltýnková and Haas, 2006; Muñoz-Antoli et al., 2006, 2008; Barragán-Sáenz et al., 2009; Kraus et al., 2014), including the worldwide distributed trematode *Echinostoma revolutum* Looss and the nematode *Parastrongylus cantonensis* (Chen) (Hai et al., 2009).

The species achieving the lowest AS-ISK scores was *Ferrissia fragilis*. This is a small basommatophoran pulmonate with a limpet-like shell typical of lentic waters often in dystrophic condition and distributed throughout Europe. Although this NIS is suspected to have been introduced in Italy (Hubendick, 1972), it has also been considered a cryptogenic species (Falkner et al., 2002). Regardless, nowadays *F. fragilis* is widespread in Italy with populations that apparently do not cause impacts to the environment. However, its distribution could be currently underestimated because of its small size and mimetic capabilities, which make it elusive (Cianfanelli et al., 2007). Overall, *F. fragilis* is not considered to pose any economic impacts and the AS-ISK outcome in the present study reflects its 'milder' bio-ecological traits.

With regard to climate change predictions, except for *G. roeselii* and *F. fragilis*, all of the other NIS achieved a score of 8 in the CCA section of AS-ISK, even though all species-specific scores were > 0. As regards the two lower-scoring species, it was estimated that climate change should not have any influence on their potential impact on biodiversity. In this respect, a recent study on the population dynamics of *G. roeselii* in a secondary semi-natural stream of the River Ticino showed that it can live in sympatry with native *E. stammeri* without causing any strong impacts (Paganelli et al., 2015). Moreover, the origin of *F. fragilis* is uncertain and, in any case, it has not been reported to cause impacts (Cianfanelli et al., 2007). On the contrary, for the other four NIS recorded in the RAA climate change could cause an increase in their density with possible consequences on biodiversity and economic impacts.

5. Conclusions

The secondary hydrographic system of a river catchment, albeit still poorly studied, represents an important source for biodiversity and is used by organisms as a pathway for dispersal. However, not only native species but also NIS can take advantage of these environments. For this reason, identification of NIS is fundamental to understand the level of bio-contamination in these areas. Implementation of long-term ecological studies on NIS is therefore crucial to unravel their role in the native communities and possible interactions with native species. As shown in the present study, the application of risk screening methods such as the SBCI index combined with the AS-ISK tool represents a first step in the quantification of the potential impact of NIS on the environment. Furthermore, except for the individual assessment of the Manila clam *Ruditapes philippinarum* (Adams and Reeve) by Copp et al. (2016), the present study is the first to apply the AS-ISK method to aquatic invertebrates, as all other applications so far have focused mainly on freshwater fish (i.e. Glamuzina et al., 2017; Li et al., 2017; Tarkan et al., 2017a,b).

Clearly, some difficulties may be encountered with the application of any risk analysis protocol. For example, the SBCI method is based on taxonomic identification at the Order level, which could therefore underestimate the presence of NIS in a community (Leuven et al., 2009; MacNeil and Briffa, 2009; Ferrario et al., 2017). Regardless, in the present study the SBCI has provided for a reliable metric by which to measure the current level of biocontamination at each of the sites under investigation. At the same time, the AS-ISK method has proven both useful and accurate in assessing NIS that are well known for causing ecological impacts or being of economic importance, especially in virtue of the availability of relevant literature-based information. On the contrary, difficulties may be encountered when applying the AS-ISK method to species that do not cause any known strong impacts or are not of economic importance, due to the paucity of information on their biological/ecological traits. The possible consequence of these difficulties would be reflected in a lower level of confidence in assessing such NIS. However, as achieved in the present study, by combining the results of an index (SBCI), which reflects the actual presence of NIS at a certain site, with those of a risk screening protocol (AS-ISK), which identifies the potential ecological and economic role of NIS, an exhaustive description was obtained on the effects that NIS could have on the local macrobenthic community. This is important for providing a first step in understanding the potential impacts of NIS in the understudied environments of the secondary hydrographic system of the River Ticino catchment. In fact, such information is essential for reliable identification and refinement of possible management strategies for conservation, also accounting for the increasingly concerning effects of predicted climate change conditions.

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